Alterations of DNA Repair in Melanoma Cell Lines Resistant to Cisplatin, Fotemustine, or Etoposide

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Resistance to chemotherapy is a common phenomenon in malignant melanoma. In order to assess the role of altered DNA repair in chemoresistant melanoma, we investigated different DNA repair pathways in one parental human melanoma line (MeWo) and in sublines of MeWo selected *in vitro* for drug resistance against four commonly used drugs (cisplatin, fotemustine, etoposide, and vindesine). Host cell reactivation assays with the plasmid pRSVcat were used to assess processing of different DNA lesions. With ultraviolet-irradiated plasmids, no significant differences were found, indicating a normal (nucleotide excision) repair of DNA photoproducts. With singlet oxygen-treated plasmid, the fotemustine- and cisplatin-resistant lines exhibited a significantly increased (base excision) repair of oxidative DNA damage. With fotemustine-treated plasmid, the fotemustine-resistant subline did not exhibit an increased repair of directly fotemustine-induced DNA damage. Similar results were obtained with cisplatin-induced DNA crosslinks in the cisplatin-resistant line. The fotemustine- and etoposide-resistant sublines have been shown to exhibit a reduced expression of genes involved in DNA mismatch repair. We used a "host cell microsatellite stability assay" with the plasmid pZCA29 and found a 2.0-fold to 2.5-fold increase of microsatellite frameshift mutations ($p \leq 0.002$) in the two resistant sublines. This indicates microsatellite instability, the hallmark of an impaired DNA mismatch repair. The increased repair of oxidative DNA damage might mediate an increased chemoresistance through an improved repair of drug-induced DNA damage. In contrast, a reduced DNA mismatch repair might confer resistance by preventing futile degradation of newly synthesized DNA opposite alkylation damage, or by an inability to detect such damage and subsequent inability to undergo DNA-damage-induced apoptosis. ***Key words: base excision repair/chemoresistance/mismatch repair/nucleotide excision repair. J Invest Dermatol 114:34–39, 2000***

Drug resistance is a major obstacle to successful treatment of metastatic malignant melanoma. Although moderate response rates (20%–50%) can be obtained by mono or poly chemotherapy, relapses are almost certain and second line therapies are largely ineffective. In other malignancies, several different mechanisms of chemoresistance have been described, such as alterations in drug transport, an increase in drug detoxification, an induction of cellular protective agents, or an increased DNA repair (of drug-induced DNA damage) (Harris, 1985). In malignant melanoma, the mechanisms of chemoresistance are largely unknown.

In order to assess the role of altered DNA repair in chemoresistant melanoma, we investigated (i) the repair of ultraviolet-induced DNA photoproducts (through the nucleotide excision repair pathway), (ii) the repair of oxidative DNA base modifications (through the base excision repair pathway), (iii) the repair of fotemustine-induced DNA adducts (through direct reversal of DNA adducts), (iv) the repair of cisplatin-induced DNA crosslinks (through DNA recombination), and (v) microsatellite stability (to assess DNA mismatch repair) in one chemosensitive parental melanoma line (MeWo) and in fotemustine-, cisplatin-, etoposide-, and vindesine-resistant MeWo sublines. These stable sublines were selected and established by continuous drug exposure for over 2 y as described by Kern *et al* (1997).

The four agents are commonly used for therapy of metastatic melanoma and represent four different modes of action. Three of the agents target DNA: fotemustine (diethyl-1-[3,2-chloroethyl]-3-nitrosoureido ethyl phosphonate), a newer chloronitrosourea, is an alkylating agent; cisplatin (cis-dichlorodiamin-platin (II)) acts through binding to DNA, principally through the formation of guanine-guanine and adenine-guanine intra- and inter-strand crosslinks (McKeage *et al*, 1991); etoposide is an inhibitor of topoisomerase II and generates DNA strand breakage (Henwood and Brogden, 1990). Only the vinca alkaloid vindesine does not interact with DNA: it binds to tubulin and inhibits its polymerization to microtubules.

We used four different "host cell reactivation assays" with the plasmid pRSVcat, damaged *in vitro* by either ultraviolet B (UVB),

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Abbreviation: MGMT, O^6-methylguanine-DNA-methyltransferase.

singlet oxygen, cisplatin, or fotemustine. This facilitated the separate functional analysis of four different DNA repair pathways. The assays in which the plasmids were damaged *in vitro* by cisplatin or fotemustine were newly established for this investigation.

Microsatellite instability, the hallmark of a DNA mismatch repair deficiency, is usually identified by polymerase chain reaction amplification of microsatellites and size comparison of resulting DNA fragments from tumor tissue and normal tissue. Such comparison is hardly possible when investigating microsatellite stability in established cell lines. Recently, we described the new shuttle vector pZCA29 for the detection of microsatellite instability by a host cell replication error assay (Diem and Rünger, 1998). Here, we used this assay to assess microsatellite instability in the melanoma lines.

MATERIALS AND METHODS

Cells The establishment and testing of drug-resistant sublines from the drug-sensitive parental cell line MeWo, derived from a lymph node metastasis, was described earlier (Kern *et al*, 1997). $MeWo_{CIS1.0}$ exhibited a 6-fold-increased relative resistance against cisplatin, compared with the parental line, $MeWo_{FOTE40}$ a 26-fold-increased resistance against fotemustine, $MeWo_{ETO1.0}$ a 35.7-fold-increased resistance against etoposide, and $MeWo_{VIND5}$ a 10.2-fold-increased resistance against vindesine. Cross-resistance has been shown for the three lines resistant to DNA damaging agents, but not with the one resistant to vindesine. Cells were cultured in Earl's modified essential medium supplemented with 10% fetal calf serum, 2 mM l-glutamine, nonessential amino acids, 1 mM pyruvate, 50 ng tylosin per ml, and 100 U penicillin/streptomycin per ml. In order to ensure maintenance of the chemoresistant phenotype, the media for the drug-sensitive sublines were supplemented with 0.25 μg cisplatin per ml, 4 μg fotemustine per ml, 0.025 μg etoposide per ml, or 0.25 ng vindesine per ml, respectively.

Two sublines have been shown to have a reduced expression of proteins involved in DNA mismatch repair. Northern blot analysis revealed an 80% reduced expression of hMLH1 in $MeWo_{ETO1.0}$ and a 70% reduced expression of hMSH2 and 50% reduced expression of hMSH1 in $MeWo_{FOTE40}$ (Lage *et al*, 1999). Here, these two lines were tested for microsatellite instability, the hallmark of a deficient DNA mismatch repair. Only subconfluent cells at exponential growth phase were used.

Host cell reactivation assay of UVB-induced DNA damage This assay was carried out as described previously (Seidman *et al*, 1985; Rünger *et al*, 1995). The 5 kb, nonreplicating plasmid pRSVcat was irradiated with 1 kJ per m^2, 5 kJ per m^2, or 10 kJ per m^2 UVB (Phillips, Hamburg, Germany; TL21 lamps, emission maximum at 315 nm, spectrum 275–365 nm) at 0°C and at a concentration of 30 μg per ml. Eight micrograms of plasmid were used to transfect 4 million cells using the electroporation procedure (Gene Pulser, BioRAD, Hercules, CA: 280 V, 960 μF capacitor, 400 μl of serum-free medium, room temperature, time constant between 20 and 30 ms). The cells were then transferred to 15 ml of prewarmed medium. At peak expression after 3 d, a cell extract was produced by three freeze-thaw cycles with ethanol in dry ice and subsequent rewarming to 37°C. Chloramphenicol acetyl transferase (CAT) activity in the cell extract, which depends on successful repair of the UVB-induced photoproducts prior to CAT expression, was determined using the one-vial procedure described by Neumann *et al* (1987) and Eastman (1999). The CAT activity was calibrated with several concentrations of CAT enzyme (Boehringer Mannheim, Germany). The values were corrected by subtracting the cell-specific background activity and were related to protein content, using the Coomassie blue method (BioRAD). The mean specific activity with undamaged plasmid was 0.2 U CAT per mg protein with MeWo. The relative CAT activity with the UV-irradiated samples, which reflects repair of DNA photoproducts on the plasmid by the host cells, was calculated as a percentage of the parallel control sample transfected with untreated plasmid.

Host cell reactivation assay of singlet oxygen-induced DNA damage As described earlier (Rünger *et al*, 1995), the plasmid pRSVcat was treated with singlet oxygen, generated by the photosensitizer methylene blue (10 μg per ml), and exposure to visible light (1, 5, and 10 min illumination with a 1000 W Osram halogen lamp, 47 W per m^2, emission between 400 and 800 nm). The plasmid concentration during treatment was 625 μg per ml. The DNA damage profile induced by this procedure comprises mainly formamidopyrimidine-DNA glycosylase (FPG)-sensitive lesions and very few single-strand breaks, pyrimidine dimers, or apurinic/apyrimidinic sites (Müller *et al*, 1990). It is identical to the DNA damage profile produced by $NDPO_2$, a chemically clean source of singlet oxygen. One FPG-sensitive lesion was generated on 2000 bp per minute of illumination. Following this plasmid treatment, the host cell reactivation assay was identical to the one with UVB-irradiated plasmid.

Host cell reactivation assay with cisplatin-induced DNA damage An almost identical assay has been described by Dabholkar and Reed (1992). The plasmid pRSVcat was treated with 1 μg per ml, 3 μg per ml, 5 μg per ml, or 10 μg per ml cisplatin for 2 h at 37°C in the dark and purified by alcohol precipitation. Neutral and alkali agarose gel electrophoresis of the treated plasmid did not detect cleavage. Following this plasmid treatment, the host cell reactivation assay was identical to the one with UVB-irradiated plasmid.

Host cell reactivation assay with fotemustine-induced DNA damage The plasmid pRSVcat was treated with 25 μg per ml, 50 μg per ml, 100 μg per ml, or 200 μg per ml fotemustine for 2 h at 37°C and purified by alcohol precipitation. Neutral agarose gel electrophoresis of the treated plasmid detected modest cleavage with the two highest doses, as shown by an increasing, but not complete, loss of supercoiled plasmid, and an increase in the relaxed circular form. Correspondingly, alkali agarose gel electrophoresis showed a partial loss of circular forms and an increase of linear plasmid. Following this plasmid treatment, the host cell reactivation assay was identical to the one with UVB-irradiated plasmid.

Host cell microsatellite instability assay This assay with the plasmid pZCA29 has been described earlier (Diem and Rünger, 1998). The plasmid carries the lacZ gene, interrupted by one $(CA)_{14}$ and one $(CA)_{15}$ repeat. These inserts inactivate lacZ by a +1 frameshift. Four million melanoma cells were transfected with 4 μg of pZCA29 as described above for the plasmid pRSVcat and incubated for 3 d. Replicated plasmids were recovered and nonreplicated plasmids were removed by digestion with the restriction enzyme *DpnI*. Recovered plasmid was introduced into *Escherichia coli* DH10B by electrotransformation (Gene Pulser, BioRAD: 2.5 kV, 25 μF capacitor, 600 Ω, total volume 80 μl, time constant between 11 and 14 ms). The bacteria were plated on selective LB agar dishes containing 100 μg per ml ampicillin for the selection of plasmid-containing bacteria (the plasmid carries the ampicillin-resistance gene) as well as 9 mg isopropyl-β-D-thiogalactopyranoside and 3.6 mg 5-bromo-4-chloro-3-indolyl-β-D-galactoside per plate for the detection of β-galactosidase activity, and incubated at 37°C overnight. Blue bacterial colonies could be distinguished from white ones. At least three independent bacterial transformations were performed with every sample of recovered plasmid DNA. Based on our previous sequence analysis of recovered pZCA29, we know that mutations in plasmids leading to a normal reading frame or to a −1 frameshift cause blue coloration. Eighty-nine percent of blue colonies were shown to contain deletions or insertions of one or two CA dinucleotides. Deletions or insertions of three CA dinucleotides do not change the reading frame and therefore are not detectable. The total number of bacterial colonies and the number of blue colonies were counted. The mutation frequency, which reflects the instability of the CA repeats in the plasmid during replication by the host cells, was calculated as the total number of blue colonies divided by the total number of all bacterial colonies. Student's t test was used to test for differences.

RESULTS

Repair of UVB-induced DNA damage (DNA photoproducts) **Table I** lists the host cell reactivation of UVB-induced DNA damage during passage of UVB-irradiated plasmid pRSVcat through the drug-sensitive parental MeWo line and the four drug-resistant sublines of MeWo. No significant differences were noted between the melanoma lines, indicating that there are no pronounced deficiencies or increases of nucleotide excision repair of DNA photoproducts in these lines. The data are based on 10 independent samples with the parental MeWo line and on two to four independent samples with the drug-resistant lines.

Repair of singlet-oxygen-induced DNA damage (oxidative base modifications) A markedly and significantly increased repair of oxidative DNA damage was found in the fotemustine-resistant and the cisplatin-resistant MeWo sublines (**Fig 1**); e.g., with 12.5 FPG-sensitive lesions/plasmid, the host cell reactivation in $MeWo_{FOTE40}$ was 2.5-fold higher and in $MeWo_{CIS1.0}$ 2.2-fold

Table I. Repair of UVB-induced DNA damage on the plasmid pRSVcat by a drug-sensitive melanoma line (MeWo) and drug-resistant sublines of MeWo, as measured with a host cell reactivation assay

	Irradiation with UVB			
	0 kJ per m^2	1 kJ per m^2	5 kJ per m^2	10 kJ per m^2
MeWo (drug-sensitive, parental)	100.0	97.9 ± 4.5%[a]	80.8 ± 20.4%	57.7 ± 23.2%
$MeWo_{FOTE40}$ (fotemustine-resistant)	100.0	95.4 ± 6.6%	66.5 ± 18.5%	41.0 ± 10.0%
$MeWo_{CIS1.0}$ (cisplatin-resistant)	100.0	87.2 ± 12.0%	61.3 ± 2.1%	51.1 ± 9.5%
$MeWo_{ETO1.0}$ (etoposide-resistant)	100.0	96.9 ± 4.4%	96.1 ± 5.5%	68.9 ± 3.6%
$MeWo_{VIND5}$ (vindesine-resistant)	100.0	91.8 ± 16.5%	95.5 ± 5.3%	66.6 ± 22.6%

[a]Relative CAT activity (as percentage of unirradiated control) in cell extracts after transfection of the plasmid pRSVcat irradiated with UVB (mean ± SD). The reactivation of the plasmid reflects the capacity of these host cells to repair UVB-induced DNA photoproducts.

higher than in the parental MeWo line ($p < 0.001$). The difference was less pronounced (1.7-fold) with the etoposide-resistant line and only minimal (n.s.) with the vindesine-resistant line. The data are based on 10 independent samples with the parental MeWo line and on two to three independent samples with the drug-resistant lines.

Repair of directly drug-induced DNA damage The ability of the fotemustine-resistant MeWo subline ($MeWo_{Fote40}$) to repair directly fotemustine-induced DNA damage was not significantly different from that of the fotemustine-sensitive parental MeWo line (**Table II**). This also applies to the cisplatin-resistant MeWo subline ($MeWo_{CIS1.0}$) and the repair of directly cisplatin-induced DNA damage (**Table III**). Both sets of data are based on two to four independent samples for each dose.

Microsatellite instability The mean yield of bacterial colonies with pZCA29 was 185280 per sample with the parental MeWo line, 61940 with $MeWo_{ETO1.0}$, and 137055 with $MeWo_{FOTE40}$. These numbers are high enough to allow a reliable calculation of the mutation frequencies (relative number of blue colonies) shown in **Fig 2**. $MeWo_{FOTE40}$ showed a 2.5-fold ($p = 0.001$) increased and $MeWo_{ETO1.0}$ a 2.0-fold ($p = 0.002$) increased mutation frequency compared with the parental MeWo line. This indicates a CA-microsatellite instability, the hallmark of a DNA mismatch repair deficiency, in these two lines. The data are based on the analysis of seven to eight independent samples with each cell line.

DISCUSSION

An increased repair of drug-induced DNA damage is an important mechanism of chemotherapy resistance in many human tumors (Harris, 1985). Specific repair mechanisms may deal with certain lesions (e.g., O^6-methylguanine), whereas other more general mechanisms (e.g., nucleotide excision repair) can handle a wider range of lesions.

Repair of DNA alkylation damage The chloroethylnitrosourea alkylating agents induce cytotoxic O^6-chloroethylguanine DNA lesions. These are repaired by O^6-methylguanine-DNA-methyltransferase (MGMT) by direct reversal of base damage. This DNA repair protein transfers and accepts alkyl groups from the O^6 position of guanine, and inactivates itself upon the repair of the DNA (Pegg and Byers, 1992; Friedberg *et al*, 1995).

In chinese hamster ovary cells, resistance to chloroethylnitrosourea was shown to occur with high acitivity of MGMT (Ludlum, 1997). Inhibition of this enzyme could restore sensitivity. Introducing a retroviral vector expressing the human MGMT gene into murine bone-marrow-derived cells with an increase of MGMT activity was shown to confer resistance to cytotoxic effects of the chloroethylnitrosourea 1,3-bis(2-chloro-ethyl)-1-nitrosourea (Maze *et al*, 1996). In lymphocytes from patients with chronic lymphatic leukemia a high rate of removal of O^6-ethylguanine was associated with an increased resistance against *N*-ethyl-*N*-nitrosourea, 1,3-bis(2-chloro-ethyl)-1-nitrosourea, and mafosfamide. In myeloid leukemic cells nitrosourea resistance could be abrogated by inactivation of MGMT by the suicide substrate O^6-methylguanine (Gerson and Trey, 1988). Wedge *et al* demonstrated that resistance to temozolamide depended upon MGMT activity in melanoma lines both *in vitro* (Wedge *et al*, 1996) and *in vivo* (Wedge *et al*, 1997).

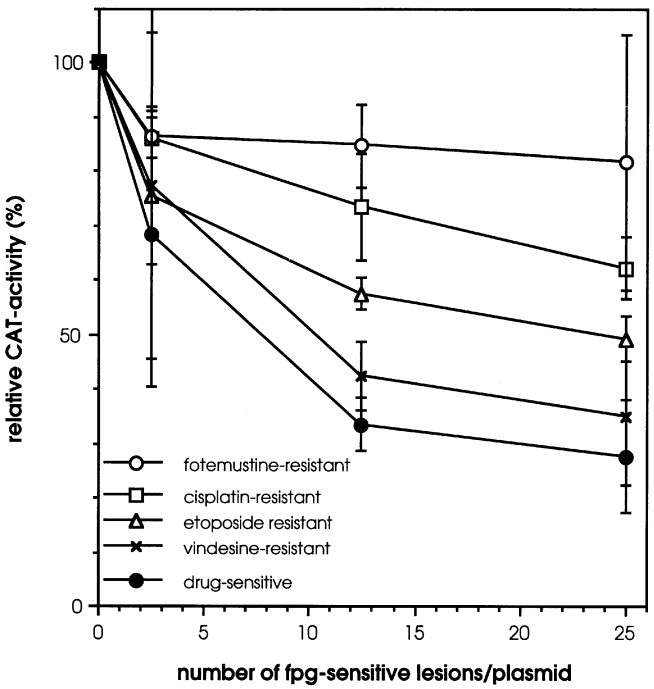

Figure 1. Repair of singlet oxygen-induced DNA damage by a drug-sensitive melanoma line (MeWo) and drug-resistant sublines of MeWo. The given numbers of oxidative base modifications were assessed as FPG-sensitive lesions. The relative CAT activity (as a percentage of untreated control) in cell extracts reflects the ability of the host cells to repair oxidative DNA base modifications (host cell reactivation assay, mean ± SD).

Here, we used a functional assay and investigated the repair of directly fotemustine-induced DNA alkylation damage on the plasmid pRSVcat by the host cell's repair enzymes. No difference was noted between the fotemustine-sensitive MeWo line and the fotemustine-resistant subline, indicating that fotemustine resistance is not due to an increased repair of DNA alkylation damage by MGMT. This result suggests that the 80- to 110-fold increased expression of MGMT described by Lage *et al* (1999) in the same fotemustine-resistant subline does not lead to an improved repair of directly fotemustine-induced DNA damage and should not be the mechanism of fotemustine resistance. This exemplifies the power of

Table II. Repair of fotemustine-induced DNA damage on the plasmid pRSVcat by a fotemustine-sensitive melanoma line (MeWo) and a fotemustine-resistant subline of MeWo, as measured with a host cell reactivation assay

	Fotemustine concentration at plasmid treatment *in vitro*				
	0 μg per ml	25 μg per ml	50 μg per ml	100 μg per ml	200 μg per ml
MeWo (fotemustine-sensitive)	100.0%	66.5 ± 4.2%[a]	58.7 ± 8.0%	38.4 ± 10.5%	30.2 ± 0.7%
$MeWo_{FOTE40}$ (fotemustine-resistant)	100.0%	91.3 ± 3.7%	72.5 ± 25.7%	36.0 ± 1.0%	20.1 ± 0.7%

[a]Relative CAT activity (as percentage of sham-treated control) in cell extracts after transfection of the plasmid pRSVcat treated with fotemustine for 2 h (mean ± SD). The reactivation of the plasmid reflects the capacity of the host cells to repair fotemustine-induced DNA damage (alkylation damage).

Table III. Repair of cisplatin-induced DNA damage on the plasmid pRSVcat by a cisplatin-sensitive melanoma line (MeWo) and a cisplatin-resistant subline of MeWo, as measured with a host cell reactivation assay

	Cisplatin concentration at plasmid treatment *in vitro*				
	0 μg per ml	1 μg per ml	3 μg per ml	5 μg per ml	10 μg per ml
MeWo (parental, cisplatin-sensitive)	100.0	83.4 ± 3.2%[a]	50.9 ± 10.9%	7.7 ± 4.5%	0.0 ± 0.0%
$MeWo_{CIS1.0}$ (cisplatin-resistant)	100.0	93.1 ± 9.8%	62.1 ± 9.1%	17.6 ± 1.0%	0.0 ± 0.0%

[a]Relative CAT activity (as percentage of sham-treated control) in cell extracts after transfection of the plasmid pRSVcat treated with cisplatin for 2 h (mean ± SD). The reactivation of the plasmid reflects the capacity of the host cells to repair cisplatin-induced DNA damage (crosslinks).

host cell reactivation assays as used here, because they facilitate a functional analysis of DNA repair processes.

Oxidative DNA damage, however, was repaired more efficiently in the fotemustine-resistant subline. Oxidative guanine base modifications are processed by the base excision repair pathway, with the initial action of DNA glycosylases, such as hOGG1 (Radicella *et al,* 1997). Therefore, we would like to hypothesize that fotemustine resistance could be conferred by an increased repair of a subset of fotemustine-induced DNA lesions, one that is processed by the base excision repair pathway. The weakness of host cell reactivation assays is that they do not identify which repair protein or which repair pathway is responsible for detected abnormalities. Both the pathway of direct repair of alkylation damage and the base excision repair pathway are known to be inducible (Friedberg *et al*, 1995; Grösch *et al*, 1998). A key enzyme of the latter is the AP-endonuclease, a plausible candidate for an increased repair of oxidative DNA damage; however, the list of plausible mechanisms could be extended considerably.

Repair of cisplatin-induced DNA damage (DNA crosslinks) Hill *et al* (1994) demonstrated that cisplatin resistance in testicular teratoma was associated with an elevated removal of platinum-DNA adducts and crosslinks. A cisplatin-resistant subclone of a cisplatin-sensitive ovarian cancer line exhibited a near 2-fold increased ability to repair cisplatin-induced DNA damage (Masuda *et al*, 1988). Inhibition of DNA repair by aphidicoline restored cisplatin sensitivity in the resistant line. This was also confirmed by Johnson *et al* (1944) who found an up to 2.5 times higher removal of cisplatin-induced inter-strand crosslinks in cisplatin-resistant ovarian cancer lines. Maynard *et al* (1989) used a host cell reactivation assay with a cisplatin-treated adenovirus in human cells. A nucleotide-excision-repair-deficient xeroderma pigmentosum cell line was found to be defective, as were some cisplatin-resistant ovarian cancer lines. Zeng Rong *et al* (1995) used a very similar assay with pRSVcat (the same plasmid we used) and found a correlation between repair of cisplatin-induced DNA damage in the host cell reactivation assay and the intrinisic resistance of lung cancer to cisplatin.

With our cisplatin-resistant MeWo subclone we found results similar to those with the fotemustine-resistant subline: no difference in the assay where we investigated drug-induced DNA lesions directly, but an increased repair of oxidative DNA lesions.

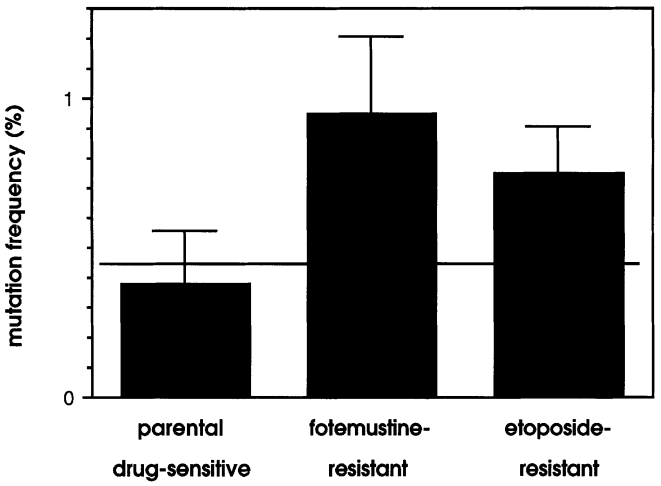

Figure 2. Microsatellite stability in a drug-sensitive melanoma line (MeWo) and drug-resistant sublines of MeWo. The mutation frequency in the plasmid pZCA29 after a 4 d passage through the host cells is indicated as the frequency of a reversion to a lacZ gene with a correct reading frame. Thus, the frequency of frameshift mutations in the CA repeat tracts inserted in the lacZ gene represents the host cells' microsatellite instability. The spontaneous mutation frequency of pZCA29 in *Escherichia coli* DH10B is shown as a horizontal line. Only mutation frequencies above this background can be attributed to the host cells' replication errors.

As with fotemustine, this might point to an increased repair of a subset of cisplatin-induced DNA lesions, e.g., of monofunctional DNA adducts, but not of bifunctional adducts (crosslinks).

Repair of UV-induced DNA photoproducts The hereditary disorder xeroderma pigmentosum exemplifies that a reduced nucleotide excision repair of DNA photoproducts increases the risk for nonmelanoma skin cancer and melanoma. Therefore, an increased repair of DNA photoproducts would not be expected in melanoma. Some of the chemotherapy-induced DNA lesions, however, are also processed by nucleotide excision repair, known to process a wide range of DNA lesions including, for example,

intra-strand crosslink. For instance, cisplatin sensitivity has been linked to a defective nucleotide excision repair, with low levels of xeroderma pigmentosum group A protein in testicular germ cell tumors (Koberle *et al*, 1999) and of XPG protein in the mouse leukemia line L1220 (Vilpo *et al*, 1995). This is why a decreased nucleotide excision repair should not be excluded as a mechanism of chemoresistance. This is in line with the findings of Hatton *et al* (1995) who demonstrated UVC-resistant subclones of metastatic melanoma with an enhanced rate of postreplication recovery, coresistance to cisplatin, and an increased repair of DNA photoproducts. In murine melanoma cells, chemosensitivity was also found to correlate with the capacity to repair UVC-induced DNA damage (and with metastatic potential) (Wei *et al*, 1997).

None of our examined MeWo sublines, however, showed a reduced nucleotide excision repair of UVB-induced DNA photoproducts.

DNA mismatch repair Unlike the DNA repair pathways mentioned above, where an increased repair has been shown to confer chemoresistance, a decreased DNA mismatch repair has been implicated in tolerance to alkylating agents (Friedberg *et al*, 1995; Karran and Hampson, 1996; Fink *et al*, 1998a). Attempts to process DNA mismatches at persistent O^6-methylguanine are thought to be made by the DNA mismatch repair pathway. In view of the strand specificity of mismatch repair for the newly synthesized strand, mismatch repair opposite the modified base would be futile, because the intact mismatch repair would continue to excise the newly synthesized DNA. This continuing futile degradation of DNA with its attendant persistence of strand breaks in DNA has been suggested to increase the potential of cell killing in mismatch-repair-proficient cells and of cell survival in mismatch-repair-deficient cells. In addition, the inability to detect DNA damage (through mismatches) in mismatch- repair-deficient cells would also impair their ability to activate apoptosis, thereby increasing cell survival, but also mutability. This model explains how cells that are incapable of mismatch repair could become tolerant to alkylating agents; however, the possibly resulting hypermutability would contribute to tumor diversification, an undesirable effect in tumor therapy.

In the fotemustine-resistant MeWo subline, we found an increased microsatellite instability. This points to a disturbance of the DNA mismatch repair pathway and is in accordance with the reduced expression of DNA mismatch repair genes described in this line earlier (Lage *et al*, 1999): $MeWo_{FOTE40}$ showed a 70%–50% reduction of hMSH2 and hMSH1 RNA levels. In accordance with the above-mentioned model, the reduced mismatch repair is suggested to be responsible for the tolerance to the alkylating agent fotemustine in this subline.

In addition to alkylating agents, topoisomerase II inhibitors have also been identified as agents for which loss of DNA mismatch repair causes drug resistance (Fink *et al*, 1998b). Therefore the impaired mismatch repair in $MeWo_{ETO1.0}$, shown here by microsatellite instability and by Lage *et al* (1999) by an 80% reduction of hMLH1 RNA levels, is suggested to be responsible for the tolerance to etoposide.

Recognition of an increased repair of chemotherapy-induced DNA damage (or of a decreased DNA mismatch repair) as a mechanism of chemoresistance in melanoma offers the outlook to overcome resistance by blocking the corresponding repair pathway. This approach has already been taken by blocking MGMT with a suicide substrate (Maze *et al*, 1996; Kurpad *et al*, 1997). In melanoma, this would require the exact identification of the involved pathway first. Therefore, the four different host cell reactivation assays presented here, which assess the processing of relatively well defined DNA lesions introduced *in vitro*, and the host cell microsatellite instability assay are suggested to be suitable tools toward that goal.

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